Kidney International, Vol. 54 (1998), pp. 1344–1350

Effects of estrogen replacement therapy on the lipoprotein profile in postmenopausal women with ESRD

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Background. Patients with ESRD have excessive cardiovascular morbidity and mortality. In postmenopausal women with normal renal function, estrogen replacement therapy decreases cardiovascular mortality by 50%, in part because of their beneficial effects on the lipoprotein profile. Because of similarities in the lipoprotein profile between healthy, postmenopausal women, and women with ESRD, we examined the effects of estrogen replacement on lipoproteins in 11 postmenopausal women with ESRD.

Methods. In a randomized, placebo-controlled crossover study (8 week treatment arms) using 2 mg daily of oral, micronized estradiol, 11 postmenopausal women with ESRD were treated. Neither baseline lipid nor lipoprotein abnormalities were used as entry criteria for study participation.

Results. Blood estradiol levels were 19 ± 4 with placebo and 194 ± 67 pg/ml ($P = 0.024$) with estradiol treatment. Total HDL cholesterol concentrations increased from 52 ± 19 mg/dl to 61 ± 20 mg/dl (16%), with placebo and estradiol treatments, respectively ($P = 0.002$). Apolipoprotein A_1 increased by 24.6% ($P = 0.0002$) with estradiol intervention. HDL_2 concentrations were 19 ± 13 with placebo and 24 ± 16 with estradiol treatment ($P = 0.046$). There were no differences in total or LDL cholesterol, other lipoprotein fractions including Lp(a), and triglycerides with 2 mg daily estradiol treatment. No significant side effects were observed.

Conclusions. Therefore, using standard dosage regimens for estrogen replacement therapy in postmenopausal women with ESRD, HDL cholesterol is increased to an extent that would be expected to improve their cardiovascular risk profile. Further studies are needed to assess whether estrogen replacement therapy decreases the incidence or severity of cardiovascular disease in ESRD patients to a similar degree compared with other women.

In contrast to age- and sex-matched controls with normal renal function, cardiovascular morbidity and mortality are excessive in patients with end-stage renal disease (ESRD) [1–3]. Arguably, patients with ESRD undergo accelerated atherosclerosis [4–6], which is a consequence of deleterious qualitative and quantitative lipid abnormalities that accompany renal insufficiency [7–9]. Common lipid abnormalities that are of potential clinical relevance for patients with renal failure are: (*1*) diminished high density lipoprotein (HDL) cholesterol and apolipoprotein A_1 concentrations; (*2*) normal or elevated low density lipoprotein (LDL) concentrations; (*3*) hypertriglyceridemia with increased very low density lipoproteins (VLDL); and (*4*) increased lipoprotein (a) [Lp(a)] concentrations [6–13]. However, with the notable exception of Lp(a), which is an independent risk factor for atherosclerotic vascular disease in patients with normal renal function [14–18] and ESRD [12], most investigators have had difficulty establishing a correlation between the lipoprotein profile and cardiovascular events in ESRD patients [1, 19, 20].

Reproductive hormones such as estrogen have a profound influence on the lipoprotein profile of both men and women. Supportive evidence for the role of sex hormones in lipid metabolism is derived from the observation of gender differences in LDL and HDL levels with age [21, 22], the effect of the menstrual cycle on plasma LDL and HDL concentrations [23, 24], and the effect of oral estrogens or progestins on the lipoprotein profile of men with prostate cancer and postmenopausal women [25–29]. For example, postmenopausal women have higher total and LDL cholesterol concentrations, and lower HDL cholesterol levels, than premenopausal women. Postmenopausal women also have an increased rate of cardiovascular events. The deleterious lipid profile and cardiovascular outcome has been ascribed to the menopausal loss of estrogen [20, 21, 30, 31]. In contrast, postmenopausal women receiving estrogen replacement therapy have half the relative risk of death due to cardiovascular disease as do postmenopausal women who do not use estrogen. Some investigators have advocated that the cardiovascular risk

Key words: cardiovascular disease, lipoproteins, cholesterol, estrogen, lipids, end-stage renal disease, menopause.

Received for publication January 13, 1998
and in revised form April 22, 1998
Accepted for publication April 23, 1998

reduction from estrogen replacement therapy is a consequence of their effect on the deleterious lipoprotein profile of being postmenopausal [32–34]. Specifically, estrogen decreases LDL cholesterol by augmenting hepatic LDL catabolism. HDL cholesterol is increased, especially HDL_2. Other quantitative and qualitative effects of estrogen include: increases in apolipoprotein A_1 in HDL, but not A_2; increased triglyceride in VLDL; and reduced Lp(a), both total and isoform specific [20, 25, 27].

It is unclear how vigorously to treat hyperlipidemia in ESRD [6–8]. The frequency and severity of complications associated with the long term use of some conventional lipid lowering agents in patients with ESRD may render their preemptive, routine use unwise [8]. Based on the effects of estrogen replacement therapy on the concentrations and metabolism of plasma lipoproteins in healthy postmenopausal women, the correction in the profile of lipoproteins is remarkably selective for the pathobiologic pattern of lipid abnormalities seen in patients with ESRD. Therefore, the use of estrogen replacement may be a novel preemptive pharmacological intervention for the lipid abnormalities in women with ESRD. We structured this study to address the hypothesis that estrogen replacement therapy will favorably improve the lipoprotein profile in postmenopausal women with ESRD undergoing maintenance hemodialysis.

METHODS

This study was approved by the Human Research Committee of the Brigham and Women's Hospital. All subjects provided informed consent. Eligible subjects were women $\geq$ 45 years old who had undergone hemodialysis for $\geq$ six months, and no menstrual periods for $\geq$ one year. Inclusion criteria were stable hematocrit $\geq$ 22%, normal hepatic transaminases, and a follicle stimulating hormone greater than 40 IU/ml. Exclusion criteria were a history of a cerebrovascular accident, breast cancer, or current diabetes mellitus requiring insulin therapy. Neither baseline lipid nor lipoprotein abnormalities were used as entry criteria for study participation. No patient was on estrogen replacement therapy for six weeks prior to entry into the study.

Study protocol

All subjects underwent an initial history and physical examination, including a screening mammogram. Seven subjects entered a lead-in study evaluating the estradiol levels attained on 1 mg oral estradiol daily for four weeks. Because of concerns about the achievement of excessive sex hormone levels, and a desire to minimize patient risks secondary to excessive estrogen exposure, the lead-in study was performed to assess the impact of a lower dose of estrogen replacement therapy. After completing the four week lead-in study, the subjects took medroxyprogesterone acetate, 10 mg daily for one week and then underwent a four week wash-out period. Medroxyprogesterone acetate was included in this and the subsequent protocol to counteract the uterine effects of unopposed estrogen stimulation. The dose and duration of medroxyprogesterone acetate therapy were conventional [25]. Based on these results, thirteen subjects entered a double-blinded cross-over study using oral micronized estradiol (2 mg) and a placebo packaged in identical blisterpacks (supplied by Mead-Johnson, Bristol Myers-Squibb). The women were initially randomized to receive estrogen daily (or placebo) for eight weeks, and after a wash-out period of four weeks, they received the other therapy for eight weeks. Prior to receiving the first intervention, subjects who had not participated in the lead-in study took medroxyprogesterone acetate, 10 mg daily for one week, and then began the first treatment arm four weeks later. After eight weeks of treatment with estrogen or placebo, all subjects took medroxyprogesterone acetate, 10 mg daily for one week. Fasting blood samples were obtained: (*1*) two days during the week prior to the first intervention (estrogen or placebo), baseline samples, (*2*) two days during the fourth week of the intervention, and (*3*) three days during the last (eighth) week of the intervention. After completion of the second treatment arm, women with intact uteri took medroxyprogesterone acetate, 10 mg daily for one week.

Blood drawing, sample processing, and assays

All blood samples were drawn from the hemodialysis angioaccess at the time of routine predialysis blood drawing with the subject fasting overnight. Samples were refrigerated, centrifuged at 2,500 rpm, aliqotted, and stored at -70°C. Estradiol was assayed using the immunofluorescent Delphia system (Wallac, Gaithersburg, MD, USA). The intraassay coefficient of variation for estradiol concentration is 10% from 0 to 99 pg/ml, 4.5% from 100 to 299 pg/ml, and 2.7% at $\geq$ 300 pg/ml. The interassay coefficient of variation is 4.6% from 0 to 300 pg/ml, 2.7% from 301-1000 pg/ml and 5.3% at $>$1000 pg/ml. There is $<$1% cross-reactivity with estrone. Estrone was assayed by radioimmunoassay using a commercial kit from Wein (Succasunna, NJ, USA). The lower limit of sensitivity is 20 pg/ml. At mean estrone levels of 80, 202 and 466 pg/ml the mean intraassay coefficients of variation are 8.4%, 5.6%, and 6.2%, respectively. At mean estrone levels of 53, 205 and 497 pg/ml, the mean coefficients of variation were 13.5%, 5.6% and 2.6%, respectively. Sex hormone binding globulin (SHBG) and serum albumin concentrations (BCG method) were measured by conventional means. Estrone, albumin and SHBG concentrations were measured on samples pooled from the three samples drawn the last week of each eight week treatment arm.

Blood samples from each subject were analyzed in one batch at the end of the study in the Lipid Research Laboratory of the Nutrition Department, Harvard School of Public Health (Boston, MA, USA). This study was standardized for cholesterol and HDL measurements as

specified by the Centers for Disease Control and the National Heart, Lung and Blood Institute. Samples from each blood draw were measured separately, and the results from each week (that is, results of three blood draws during week 8) were averaged. VLDL was prepared from plasma by overlaying 0.5 ml of 0.9% sodium chloride over 0.5 ml of plasma, and spinning in a Beckman Type 25 rotor (Beckman Instruments, Palo Alto CA, USA) at 25,000 rpm for 16 hours. The VLDL fraction was separated from the LDL and HDL fractions by tube slicing. HDL and HDL_3 were sequentially separated by precipitation with dextran sulfate and magnesium chloride [35]. HDL_2 was obtained by subtraction of HDL_3 from the total HDL. Cholesterol and triglycerides were measured with enzymatic reagents and quantified photometrically using a COBAS Mira Plus auto-analyzer (Roche Diagnostics Systems, Belleville, NJ, USA). Cholesterol, VLDL, the combined LDL and HDL fractions, HDL and HDL_3 were measured in whole plasma. Triglycerides and apolipoprotein B were measured in whole plasma and in the VLDL fraction. Apolipoprotein B, apolipoprotein A_1 and Lp(a) were measured by immunoturbidimetry with rabbit antiserum, [36] obtained from Incstar (Stillwater, MN, USA) using the COBAS Mira Plus auto-analyzer. The coefficients of variation for blinded control specimens were as follows: 2.4% for cholesterol, 3.3% for HDL cholesterol, 2.8% for HDL3 cholesterol, 2.2% for apolipoprotein A_1, 2.0% for apolipoprotein B, and 3.0% for Lp(a).

Statistical analysis

Duplicate measurements were averaged and weighted as a single test. Pre- and post-estrogen exposure lipoprotein and hormone parameters were compared using the Wilcoxon rank sum test. Comparisons were considered statistically significant at $P < 0.05$. Non-parametric tests, like the Wilcoxon rank sum, tend to be far more conservative with regard to type I error than corresponding parametric tests (such as, Student's *t*-test). Analyses were conducted using SAS (The SAS Institute, Cary, NC, USA).

RESULTS

The principal rationale for the lead-in study of four weeks with 1 mg micronized oral estradiol daily was to assess its impact on estrogen levels and to minimize patient risks secondary to excessive estrogen exposure, not as an intervention to effect the lipoprotein profile. Seven subjects received 1 mg oral estradiol daily. One subject withdrew because of ill health during fasting prior to blood sampling at the time of hemodialysis. The remaining six subjects completed the lead-in study, and three of these women participated and completed the blinded, crossover portion of the study. Thirteen subjects total were randomized to the blinded crossover trial, but serum samples from one subject were not able to be analyzed because of a handling mishap. Twelve subjects completed the study. One subject withdrew because she underwent cadaveric renal transplantation during the trial. Another subject experienced vaginal bleeding due to a benign vaginal lesion that was uneventfully excised; she completed the study. Demographic information on the 11 subjects who completed the trial is provided in Table 1. Their mean age was 61.4 ± 2.7 years (± SEM); eight of the women were African American, one was Hispanic, and two were white. Two women had non-insulin dependent diabetes mellitus.

Table 1. Demographic profile of subjects completing randomized trial

Subject	Age *years*	Etiology of ESRD	Length of renal replacement Rx *years*	Estimated dry weight *kg*
1	53	hypertension	4	59
2	62	polycystic kidney disease	2	54.5
3	56	hypertension	23	63
4	72	hypertension	0.7	55.5
5	45	focal glomerulosclerosis	0.5	70.5
6	59	hereditary nephritis	9.5	70
7	65	diabetes mellitus	0.5	66.4
8	75	diabetes mellitus	1.3	82
9	63	unknown	3.0	56.5
10	70	hypertension	10	57.5
11	58	hereditary nephritis	0.5	66.5

Table 2. Hormonal and lipid parameters with four weeks of estradiol

	Baseline value	Value at week 4	*P* value
Estradiol *pg/ml*	21 ± 6	91 ± 19	0.002
Total cholesterol *mg/dl*	222.7 ± 29.9	247.0 ± 32.7	0.001
Total HDL cholesterol *mg/dl*	46.5 ± 4.9	55.6 ± 5.5	0.029
LDL cholesterol *mg/dl*	45.5 ± 7.3	43.4 ± 7.2	0.549
HDL_2 cholesterol *mg/dl*	8.9 ± 2.8	12.8 ± 4.1	0.052
HDL_3 cholesterol *mg/dl*	37.6 ± 3.7	42.8 ± 2.8	0.087
Triglycerides *mg/dl*	227.4 ± 36.3	217.2 ± 36.3	0.549

Abbreviations are: LDL, low density lipoprotein; HDL, high density lipoprotein.

Results are from six women receiving 1 mg per day of estradiol

Lipoprotein profiles

Lipoprotein and estradiol measurements were obtained from the six women who completed the preliminary intervention trial during which they ingested 1 mg estradiol daily for four weeks. Table 2 provides the change in the lipid profile for these subjects. During the period of estradiol ingestion, estradiol levels increased approximately 4.5-fold. In parallel, total cholesterol, total HDL cholesterol, and the HDL_2 cholesterol fraction increased significantly. No significant change was observed in triglycerides.

Hormonal parameters from the placebo controlled, cross-over intervention trial are provided in Table 3. Higher estradiol levels were achieved with 2 mg per day of estradiol, in comparison to 1 mg daily. In comparison to eight weeks of placebo therapy, statistically significant increases in estradiol, its metabolite estrone, and the

Table 3. Hormonal levels with eight weeks of therapy

Hormonal parameter	Intervention			
	Placebo	Estradiol[a]	*P* value	Reference range
Estradiol *pg/ml*	19 ± 4	194 ± 67	0.024	<30
Estrone *pg/ml*	24 ± 6	684 ± 186	0.006	15–55
Albumin *mg/dl*	4033 ± 118	3947 ± 84	0.220	3900–5400
Sex hormone binding globulin *nmol/liter*	61 ± 7	94 ± 9	0.0006	8–85

[a] Results are from eleven women receiving 2 mg per day of estradiol

Table 4. Serum lipoprotein levels with eight weeks of therapy (mean ± SEM)

Lipoprotein *mg/dl*	Intervention			
	Placebo	Estradiol[a]	*P* value	Reference range
VLDL				
Cholesterol	22.6 ± 19.6	24.2 ± 20.8	0.643	<20
Triglycerides	78.7 ± 62.4	92.4 ± 81.2	0.319	NE[b]
Apolipoprotein B	17.0 ± 13.4	17.9 ± 13.5	0.647	NE
LDL				
Cholesterol	131.0 ± 56.2	129.1 ± 49.1	0.837	<130
Apolipoprotein B	55.9 ± 21.7	57.1 ± 17.7	0.761	NE
HDL				
Cholesterol	52.2 ± 19.4	60.6 ± 19.6	0.002	>35
HDL_2				
Cholesterol	18.5 ± 12.7	23.8 ± 15.9	0.045	NE
HDL_3				
Cholesterol	33.7 ± 9.7	36.8 ± 8	0.106	NE
Apolipoprotein A_1	149.7 ± 42.2	186.6 ± 39.1	0.0002	115–220
Total cholesterol	205.4 ± 71.8	211.4 ± 56.6	0.538	<200
Total triglycerides	164.1 ± 96.2	190.1 ± 103.4	0.176	<130
Lp(a)	36.8 ± 33.3	36.8 ± 29.2	0.987	NE

[a] Each of eleven women received 2 mg per day of estradiol
[b] NE, normal range has not been established

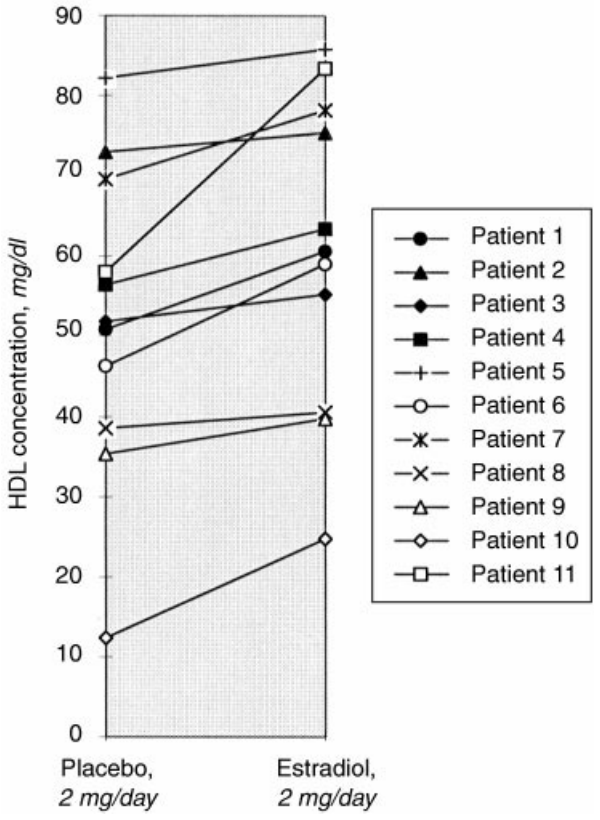

Fig. 1. Individual total HDL cholesterol concentration (mg/dl) in 11 women with completing eight weeks of placebo and with eight weeks of estradiol (2 mg per day).

steroid-inducible SHBG were observed. Total HDL cholesterol concentration increased in all subjects (Table 4 and Fig. 1A). The increase of the mean values was 16.1%. Total HDL cholesterol concentration increased from 52.2 ± 19.4 mg/dl after placebo treatment to 60.6 ± 19.6 mg/dl (mean ± SEM; $P = 0.002$) following estradiol. The fraction of HDL that accounted for the majority of the increase in total HDL cholesterol was HDL_2, which increased by 28.6% ($P = 0.046$), whereas HDL_3 cholesterol increased by 9.2%, a difference that did not reach statistical significance (Table 4). With eight weeks of estradiol treatment, nine of eleven women had increases in HDL_2 cholesterol, and eight women had increases in HDL_3 cholesterol. LDL cholesterol was unchanged for all subjects. There was no significant association between the increase in HDL cholesterol concentration and the concentrations of estradiol or estrone. Apolipoprotein A_1 increased by 24.6%, from 149.7 ± 42.2 mg/dl to 186.6 ± 39.1 mg/dl ($P = 0.0002$). Lp(a) was not significantly affected. Although the total triglyceride concentration increased by 15.8% with daily estradiol ingestion, the elevation compared to placebo therapy was not significant.

DISCUSSION

Low HDL cholesterol concentrations are often, but not uniformly observed in patents with chronic renal insufficiency, especially the HDL_2 subfraction [37]. In the present study, HDL cholesterol concentrations were within the normal range. However, oral micronized estradiol increased the serum total HDL cholesterol concentration by an average of 8.4 mg/dl (16.1%), and increased apolipoprotein A_1 by 24.6% compared with placebo treatment. Previous studies in patients with normal renal function suggest that comparable increases in total HDL cholesterol can be expected to decrease the risk of cardiovascular disease by approximately 50% [38]. Even the 1 mg daily dose of estradiol that was administered during the lead-in portion of the current intervention trial increased HDL by 20% after only four weeks. This is a level of response that would also be expected to significantly decrease the incidence of coronary artery disease. The triglyceride concentration was not significantly increased by low dose estrogen replacement therapy, but the lack of statistical significance may be due in part to the small sample size in the setting of large inter-individual variations in triglyceride concentrations. The mean triglyceride levels in this subject group were not out of the normal range, however. Hypertriglyceridemia

seen in hemodialysis patients is thought to be due to defective triglyceride removal secondary to inhibition of lipoprotein lipase activity [39]. The doses of estrogen used in the intervention reported herein had no significant effect on plasma Lp(a) or LDL cholesterol levels. Insufficient power (that is, large type 2 error) is an important consideration in interpreting any apparent lack of effect, since size (and scatter) may limit the ability to detect differences among groups.

Previous investigations have demonstrated that basal estradiol concentrations as well as those attained after oral estradiol ingestion are greater in postmenopausal women with ESRD than in body-mass index matched controls, probably due to decreased clearance of endogenous and exogenous estradiol [40]. Although no adverse effects were observed in the present study, a larger number of subjects with a longer period of estrogen ingestion is needed to validate this observation. Because of the absence of correlation between the increase in estradiol and estrone concentrations and the increase in HDL cholesterol, it is unlikely that extraordinarily high sex hormone concentrations are needed to improve the HDL:LDL ratio. In that the biologic effect of 2 mg daily of oral estradiol is not much different than that observed with 1 mg daily, but blood estradiol levels were lower with 1 mg daily oral estradiol (194 vs. 91 pg/ml for 2 vs. 1 mg/day, respectively), we advocate the use of the lower estradiol dose for future clinical intervention trials. This will minimize the risks associated with higher hormone levels.

Not all ESRD patients have abnormal lipoprotein profiles. Moreover, the incidence of death due to cardiovascular disease in the ESRD population is very high regardless of the lipoprotein profiles found. Therefore, other factors may contribute to this excessive cardiovascular mortality, and/or the lipoprotein profile is an incomplete surrogate of the deleterious effect of hyperlipidemia in ESRD. Similarly, in healthy postmenopausal women, estrogen replacement therapy is associated with a decrease in the risk of death due to cardiovascular disease by 50% [41]. However, this effect appears to be partly independent of the individuals' lipoprotein profile, and is much greater than would be predicted by the change in the lipoprotein profile alone. Furthermore, based on longitudinal studies of healthy women with angiographically demonstrable coronary artery stenosis, simply having received estrogen replacement therapy at any time reduces the risk of death by 10 to 27% in comparison to nonusers [42]. Therefore, although the change in the lipoprotein profile was moderate in this study as in studies of women with normal renal function, a similar experience in postmenopausal women with ESRD may result in substantial benefit with reductions in deaths due to cardiovascular disease.

Many treatments for hyperlipidemia have been evaluated in ESRD including gemfibrozil [43], probucol [44], pantethine [45], and LDL-pheresis [46]. Lovastatin and simvastatin treatment in ESRD patients on hemodialysis increases HDL cholesterol and apolipoprotein A_1 and decreases triglycerides [47]. However, whether these changes reduce the incidence of heart disease has not been established. Furthermore, consideration must be made of the major side effects associated with the use of many of the conventional lipid lowering agents. For example, HMG CoA reductase inhibitors have major routes of renal excretion. When used alone, but especially in combination therapy with other lipid lowering agents, there is an increased incidence of rhabdomyolysis and elevated hepatic transaminases. Similar side effects have been observed with gemfibrozil, as well as enhanced sensitivity to oral anticoagulants. Probucol is relatively unsafe in patients with conduction system abnormalities. Some data suggests that modification of dietary fat can significantly decrease triglycerides in hemodialysis patients [48]. However, if elevations of HDL cholesterol seen with gemfibrozil decreases cardiovascular risk [43], similar benefit may be achieved from the use of estrogen replacement therapy with fewer side effects. Added cardiovascular benefits from estrogens include their ability to dilate coronary arteries [49], decrease coronary resistance [50], and increase coronary perfusion.

The data presented herein indicate that the use of estrogen replacement therapy results in increases of HDL cholesterol and apolipoprotein A_1 that are comparable to those achieved in healthy postmenopausal women. In the present study, baseline lipid or lipoprotein abnormalities were not used as entry criteria. A principal reason for this approach is that in this early intervention trial, we were uncertain what benchmark abnormalities to use. Instead, we elected to examine the effect of estrogen replacement therapy on a random sample of postmenopausal women with ESRD, as was done in a previous landmark trial in healthy postmenopausal women [25]. Furthermore, the strategy of including ESRD patients, indifferent of the severity of their lipid abnormalities, permitted us to profile the type and severity of baseline lipid and lipoprotein derangements in a random sample of postmenopausal women with ESRD. The findings reported herein may not be reproducible in some postmenopausal women with an alternative lipid or lipoprotein profile. It is unknown whether patients with greater derangement in lipoprotein parameters would achieve the same, greater, or lesser effects with estrogen replacement as the subjects in the present study. Furthermore, this study has not addressed the ultimate question of whether improvements in lipoprotein parameters will lead to comparable reductions in cardiac mortality. Larger clinical trials are needed to answer these questions, and to determine whether estrogen replacement therapy should be conventional care for postmenopausal women with ESRD. In the design of future trials with estrogen replacement therapy, consideration

should be made of their potential to improve other comorbid conditions in postmenopausal women such as prevention of estrogen-deficiency related osteoporosis [51, 52], symptoms of urogenital atrophy [53], improved skin elasticity [54] and decreased wrinkling [55], and a decreased risk of Alzheimer's disease [56–59]. In patients with ESRD, platelet dysfunction is improved [60, 61]. These potential benefits must be balanced against the possible increased risks of breast cancer [62, 63], non-fatal venous thromboembolism [64], and cholelithiasis [65]. The increase risk of thromboembolic disease may be a consequence of the increase in clotting factors observed with oral, but not transdermal, estrogen replacement therapy [66, 67].

ACKNOWLEDGMENTS

This study was supported by grants from Baxter, Inc. and Mead-Johnson, Inc. Partial funding for this work (to W.F.O.) was by grant #DK520521 of the National Institute of Diabetes, Digestive, and Kidney Disease.

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